

(10mM Tris-Cl, 1mM EDTA, pH 8. 0) and concentrated using a Centricon Concentrator (Amicon). The purified gene fragments were then sequenced by an automated DNA sequencer (Applied Biosystems, model 377) using one or more of the same primers employed in PCR. The upstream primer SEQ ID NO: 1 and the downstream primer SEQ ID NO: 6 were used for sequencing the amplified products, fragment A of the human  $\alpha_{1B}$ -adrenergic receptor gene and fragment A of  $\beta_2$ -adrenergic receptor gene, respectively. As shown in Figure 5 (SEQ ID NO:11) and 6 (SEQ ID NO:12), each sequencing read approximately 550 bases. Other primers described herein including primer SEQ ID NOS: 1, 2, 3, 5, and 7 can also be used for direct sequencing with high reliability. By use of an automated sequencer and sequencing PCR products from in excess of 15 different subjects, we obtained consistent results in accordance with the published coding sequences of the human  $\beta_2$ - and exon 1 of the human  $\alpha_{1B}$ -adrenergic receptor. Repeated sequencing of PCR products of the same individuals revealed a 100% reliability of our PCR methods without requirement for repeat isolation of PCR fragments. Occasionally occurring "ambiguous reads", which are the result of a reading error of the automated sequencer, can generally be corrected afterwards without re-isolating and sequencing the PCR fragments (Figures 5 and 6).

**In the Claims:**

Please amend claims 1-24, 26, 27 and 29, as follows:

1. (Once Amended) An oligonucleotide primer pair for amplifying a human  $\alpha_{1B}$ -adrenergic receptor gene, wherein each individual primer comprises a linear sequence of at least 15 nucleotides in length that hybridizes under high stringency to the sequence shown in SEQ ID NO:9, is non-self hybridizing, has a melting temperature within the range of 50°C to 85°C; wherein each primer of said primer pair is non-cross hybridizing, wherein said primer pair anneals to two distinct regions of the human  $\alpha_{1B}$ -adrenergic receptor gene as shown in SEQ ID NO:9 which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked

by said regions in a polymerase chain reaction; and wherein at least one primer of said pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction.

2. (Once Amended) An oligonucleotide primer pair of claim 1, wherein said primer pair amplifies a fragment selected from the group consisting of region A in Figure 1, and region B in Figure 1.

3. (Once Amended) An oligonucleotide primer pair of claim 1, wherein each individual primer of said pair comprises a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:1 5'CGGGGGAAGCAAAGTTTCA3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

4. (Once Amended) An oligonucleotide primer pair of claim 1, wherein at least one primer of said pair comprises a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No: 1 5'CGGGGGAAGCAAAGTTTCA3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

5. (Once Amended) An oligonucleotide primer pair of claim 1 wherein each individual primer of said pair comprises a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

6. (Once Amended) An oligonucleotide primer pair of claim 1, wherein at least one primer of said pair comprises a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

7. (Once Amended) An oligonucleotide primer pair of claim 1 comprising the nucleotide sequences SEQ ID No:1 5'CGGGGGAAGCAAAGTTTCA3' and SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

8. (Once Amended) An oligonucleotide primer pair of claim 1, wherein at least one primer of said pair comprises the nucleotide sequence SEQ ID No:1 5'CGGGGGAAGCAAAGTTTCA3' or SEQ ID No:2 5'CGGCAGTACATGACTAGAAT3'.

9. (Once Amended) An oligonucleotide primer pair of claim 1 comprising the nucleotide sequences SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' and SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

10. (Once Amended) An oligonucleotide primer pair of claim 1, wherein at least one primer of said pair comprises the nucleotide sequence SEQ ID No:3 5'CTCTCCTTGGGTGGAAGGA3' or SEQ ID No:4 5'AGCTCATCAGTAAACCCAAG3'.

11. (Once amended) An oligonucleotide primer pair for amplifying a human  $\beta_2$ -adrenergic receptor gene, wherein each individual primer comprises a linear sequence of at least 15 nucleotides in length that hybridizes under high stringency to the sequence shown in SEQ ID NO:10, is non-self hybridizing, has a melting temperature within the range of 50°C to 85°C; wherein each primer of said primer pair is non-cross hybridizing, wherein said primer pair

anneals to two distinct regions of the human  $\beta_2$ -adrenergic receptor gene shown in SEQ ID NO:10, which are separated by a distance of at least about 400 nucleotides; wherein said primer pair has the property of yielding a substantially homogenous plurality of gene segments flanked by said regions in a polymerase chain reaction; and wherein at least one primer of said pair has the property of extending 3' end sequence complementary to a template sequence in a DNA polymerase reaction.

12. (Once Amended) An oligonucleotide primer pair of claim 11, wherein said primer pair amplifies a fragment selected from the group consisting of region A in Figure 2; and region B in Figure 2.

13. (Once Amended) An oligonucleotide primer pair of claim 11, wherein each individual primer of said pair comprises a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:5 5'GAATGAGGCTTCCAGGCGTC3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

14. (Once Amended) An oligonucleotide primer pair of claim 11, wherein at least one primer of said pair comprises a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No: 5 5'GAATGAGGCTTCCAGGCGTC3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

15. (Once Amended) An oligonucleotide primer pair of claim 11, wherein each individual primer of said pair comprises a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' or a linear

sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:8  
5'TCCTCTAGGACTAAAGCTC3'.

16. (Once Amended) An oligonucleotide primer pair of claim 11, wherein at least one primer of said pair comprises a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' or a linear sequence that hybridizes under high stringency to the polynucleotide shown in SEQ ID No:8 5'TCCTCTAGGACTAAAGCTC3'.

17. (Once Amended) An oligonucleotide primer pair of claim 11 comprising the nucleotide sequences SEQ ID No:5 5'GAATGAGGCTTCCAGGCGTC3' and SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

18. (Once Amended) An oligonucleotide primer pair of claim 11, wherein at least one primer of said pair comprises the nucleotide sequence SEQ ID No: 5 5'GAATGAGGCTTCCAGGCGTC3' or SEQ ID No:6 5'GATGATGCCTAACGTCTTG3'.

19. (Once Amended) An oligonucleotide primer pair of claim 11 comprising the nucleotide sequences SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' and SEQ ID No: 8 5'TCCTCTAGGACTAAAGCTC3'.

20. (Once Amended) An oligonucleotide primer pair of claim 11, wherein at least one primer of said pair comprises the nucleotide sequence SEQ ID No:7 5'TTCTACGTGCCCCTGGTG3' or SEQ ID No:8 5'TCCTCTAGGACTAAAGCTC3'.

21. (Once Amended) A method of amplifying a segment of a human  $\alpha_{1B}$ -adrenergic receptor gene of a subject comprising the step of:

[a] providing a biological sample of the subject containing nucleic acid molecules;

b)] amplifying a segment of the human  $\alpha_{1B}$ -adrenergic receptor gene from nucleic acid contained within a biological sample by employing an oligonucleotide primer pair of claim 1, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of segments of the gene.

22. (Once Amended) A method for identifying a genetic variation in a human  $\alpha_{1B}$ -adrenergic receptor gene of a subject comprising the steps of:

a) amplifying a segment of the a human  $\alpha_{1B}$ -adrenergic receptor gene from nucleic acid contained within a biological sample by employing an oligonucleotide primer pair of claim 1, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of amplified segments of the gene; and

b) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step.

23. (Once Amended) A method for identifying a genetic variation in a human  $\alpha_{1B}$ -adrenergic receptor gene of a subject according to claim 22, wherein the sequence analytical step is selected from the group of nucleotide sequencing, single-strand conformation polymorphism assay, allele-specific oligonucleotide hybridization, Southern blot analysis, and restriction endonuclease digestion.

24. (Once Amended) A method for diagnosing a disease associated with a genetic alteration of a human  $\alpha_{1B}$ -adrenergic receptor gene of a subject comprising the steps of:

a) amplifying a segment of the  $\alpha_{1B}$ -adrenergic receptor gene from nucleic acid contained within a biological sample by employing an oligonucleotide primer pair of claim

1, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of amplified segments of the gene;

b) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step; and

c) determining a correlation of the detected variation between the subject and a control.

26. (Once Amended) A method of amplifying a segment of a human  $\beta_2$ -adrenergic receptor gene of a subject comprising the step of:

a) amplifying a segment of the human  $\beta_2$ -adrenergic receptor gene from nucleic acid contained within a biological sample by employing an oligonucleotide primer pair of claim 11, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of segments of the gene.

27. (Once Amended) A method for identifying a genetic variation in a human  $\beta_2$ -adrenergic receptor gene of a subject comprising the steps of:

a) amplifying a segment of the human  $\beta_2$ -adrenergic receptor gene from nucleic acid contained within a biological sample by employing an oligonucleotide primer pair of claim 11, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of segments of the gene; and

b) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step.

29. (Once Amended) A method for diagnosing a disease associated with a genetic alteration of a human  $\beta_2$ -adrenergic receptor gene of a subject comprising the steps of:

a) amplifying a segment of the human  $\beta_2$ -adrenergic receptor gene encoding said receptor from nucleic acid contained within a biological sample by employing an

oligonucleotide primer pair of claim 11, a primer-dependent DNA polymerase, and a sufficient amount of deoxyribonucleotides to generate a plurality of amplified segments of the gene;

- b) identifying a sequence variation of the resulting amplified products relative to a control using at least one sequence analytical step; and
- c) determining a correlation of the detected variation between the subject and a control.

Please add new claims 37-38, as follows:

37. (New) The oligonucleotide primer pair of claim 1 wherein said linear sequence is at least about 18-23 nucleotides in length.

38. (New) The oligonucleotide of claim 11 wherein said linear sequence is at least about 18-23 nucleotides in length.